Late presenting atypical severe combined immunodeficiency (SCID) associated with a novel missense mutation in DCLRE1C

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1 Table and 1 Figure

To the Editor:

Immunodeficiency associated with mutations in the DNA cross-link repair 1C gene (DCLRE1C) can have variable clinical presentations including severe combined immunodeficiency (SCID), Omenn syndrome, atypical SCID or common variable immunodeficiency (CVID) (1)(2)(3). DCLRE1C encodes the protein Artemis, a nuclease with intrinsic 5′-3′ exonuclease activity on single-stranded DNA that is involved in non-homologous end joining (NHEJ). Artemis is essential for V(D)J recombination of the immunoglobulin and T-cell receptor genes that occur during B- and T-cell development. Biallelic null mutations in DCLRE1C, completely ablating the function of Artemis, typically leads to T-, B-, radiosensitive SCID presenting in infancy, while biallelic hypomorphic mutations can cause a more variable clinical presentation that may present later during childhood or even in adults (1). We evaluated a five-year-old boy presenting with cytomegalovirus (CMV) pneumonia and long-term viral gastroenteritis. The patient was the son of consangious parents and first presented at 3 months post partum with upper respiratory tract infection and intermittent diarrhea, where the symptoms recurred in bouts during the following 5 years. He was evaluated for celiac disease, inflammatory bowel disease and food allergies without a conclusive diagnosis. The boy had normal psychomotor development and there was no failure to thrive, despite the recurrent symptoms.

At the time of our evaluation the patient had developed a febrile illness with cough and worsened diarrhoea. Chest CT showed bilateral ground glass opacities and PCR detected 43 000 copies/mL of CMV in bronchoalveolar lavage, with no other significant pathogens being identified. Simultaneous PCR of stool samples detected norovirus, enterovirus, adenovirus and astrovirus. CMV serology showed evidence of previous infection and the CMV pneumonitis was interpreted as a reactivation.

Peripheral T-cell numbers were diminished and the proportion of double negative T-cells (CD3+CD4-CD8-) was elevated with at 20% of the peripheral T-cell population (Table 1). T-cell responses to mitogens and recall responses to selected microbial antigens as evaluated by our clinical FASCIA method were impaired in the CD4+ T-cell subsets with the exception of responses to staphylococcal enterotoxin A which were normal (Table 1)(4). The peripheral B-cell population was reduced and showed a decreased level of naïve B-cells (IgD+ CD27-) and an increased proportion of switched memory B-cells (IgD-, CD27+) (Table 1). Serum concentrations of IgG and IgM were normal including IgG subclasses, while IgA was not detectable in serum (Table 1). Specific antibodies to several vaccine antigens (tetanus, diphtheria and pneumococcus) were also within the normal range (Table 1).

The intriguing clinical picture and immunologic findings prompted us to perform whole-exome sequencing (WES). We identified a novel homozygous missense mutation in DCLRE1C. The mutation (c.272G>T, p.G91V) localized to the β-lactamase domain of Artemis, which is essential for its enzymatic activity (5). The mutation was predicted to be deleterious by several variant evaluation metrics (CADD score 34, Sift 0.9122) and the affected residue is conserved in several vertebrate species (phyloP100way 0.8579). The variant has previously not been described in the literature or in the ExAc and 1000 genomes databases. Confirmatory sanger sequencing revealed that the patient's parents were both heterozygous carriers. In order to evaluate the effect of the mutation on V(D)J recombination, the patients TCR repertoire was analysed by spectratyping of 24 Vβ, 12 Vδ and 9 Vɣ families (Figure 1). Compared to a healthy control analysed simultaneously the patients Vβ repertoire was aberrant with a non-Gaussian distribution across the majority of Vβ families and a disturbed appearance of the δ and ɣ families. These findings indicate that the patients mutations in DCLRE1C gives rise to an Artemis protein that is unable to fully assist in V(D)J recombination and thus leads to an impaired TCR diversity, resulting clinically in atypical SCID. Assessing the impact of novel mutations in DCLREC1C on radio-sensitivity is clinically important, as increased radio sensitivity will affect a patients response to alkylating chemotherapy which is often used in the conditioning regiment of allogeneic hematopoietic

stem cell transplantation (HCT)(6). We therefore cultured fibroblasts obtained by skin biopsy from the patient and exposed them to varying amounts of ionizing radiation. The patient's fibroblasts showed a marked increase in radio-sensitivity compared to fibroblasts from a healthy donor (Table 1), indicating compromised DNA repair.

The patients CMV pneumonia responded to treatment with ganciclovir. His gastroenteritis also improved during the following weeks with lessened stool frequency and volume. A decision was made to perform HCT based on the clinical picture with progressive immunodeficiency and inability to control latent CMV in conjunction with immunologic findings indicating atypical radiosensitive SCID. The patient was conditioned with reduced dose fludarabin (150 mg/m^2) and treosulfan (21g /m^2), combined with antithymocyteglobulin (5 mg/kg). Due to lack of HLA-matched donors, an *in vitro* TCR alpha/beta positive T-cell depleted peripheral blood stem cell graft from the patient's haploidentical father was used. The patient had an uneventful post-HCT course and is now in good clinical condition beyond the 2-year follow-up.

In conclusion, we present a novel c.272G>T variant in DCLREC1C, associated with a radiosensitive atypical SCID with clinical signs of progressive T-cell deficiency. The patient had a restricted oligoclonal TCR repertoire associated with susceptibility to several viral infections. Surprisingly however the patient, despite a somewhat abnormal peripheral B-cell phenotype, had normal IgG levels including antibodies to vaccine antigens, indicating functional B-cell immunity. Additionally, the patient has no history infections with encapsulated bacteria, which further supports the presence of functional B-cell immunity. Taken together our findings associates the c.272G>T variant with atypical radiosensitive SCID.

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Table 1

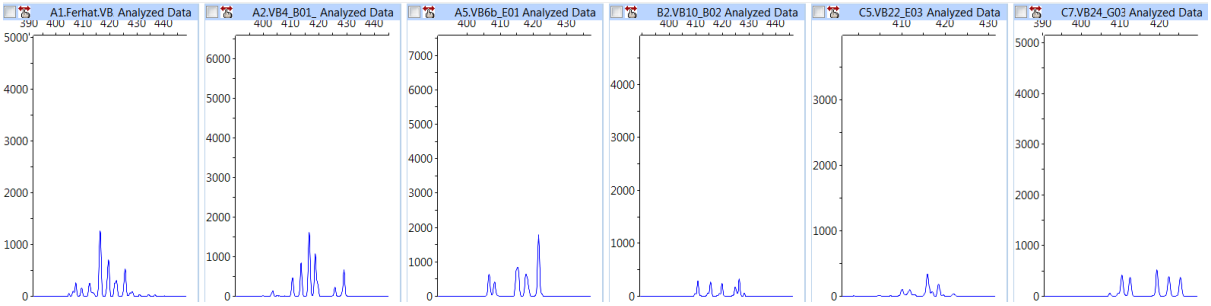
Patient	Normal range	
Peripheral cell numbers		
CD3+ T-cells	360 cells/µl	700-4200 cells/µl
CD3+ CD4+ T-cells	80 cells/µl	300-2000 cells/µl
CD3+ CD8+ T-cells	150 cells/µl	300-1800 cells/µl
CD3+ CD8- CD4- DN-T-cells	20%	2-9%
CD56+ NK-cells	1520 cells/µl	90-900 cells/µl
CD19+ B-cells	90 cells/µl	200-1600 cells/µl
IgD+ CD27- (Naïve)	16%	76-85%
IgD- CD27+ (Switched)	34%	5-12%
T-cell activation by FASCIA		
SEA CD4+ T-cells	1951 blasts/ µl	553-7743 blasts/ µl
PWM CD4+ T-cells	85 blasts/ µl	233-2189 blasts/ µl
ConA CD4+ T-cells	99 blasts/ µl	620-3800 blasts/ µl
PPD Tb CD4+ T-cells	0 blasts/ µl	11-2022 blasts/ µl
Pnc CD4+ T-cells	0 blasts/ µl	0-269 blasts/ µl
Serum immunoglobulins		
IgG	13.6 g/L	6.1-14.5 g/L
IgA	<0.08 g/L	0.50-2.70 g/L
IgM	0.52 g/L	0.27-1.50 g/L
Serum antibodies		
Tetanus (IgG)	3.2 IE/mL	0.09-13 IE/mL
Diphtheria (IgG)	0.065 IE/mL	>0.01 IE/mL
Pneumococcus (IgG)	53 mg/L	9.2-230 mg/L
Radio-sensitivity of primary fibroblasts		**HC**
No radiation	100%	100%
1 G	43%	94%
3G	14%	19%
6G	4%	5%

Table 1

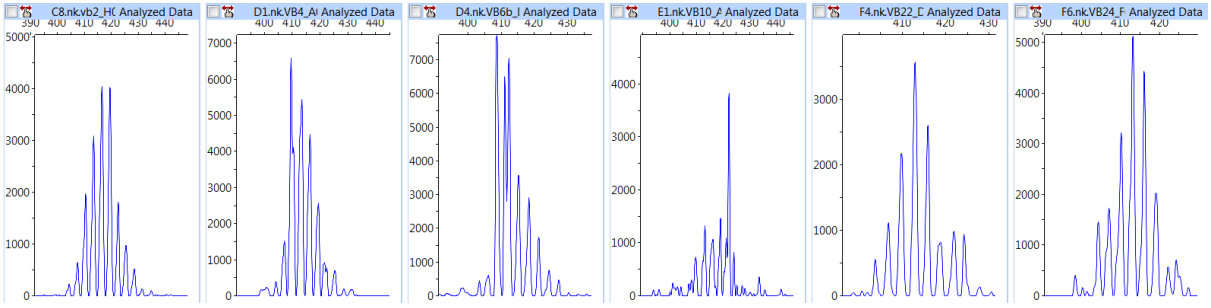
Laboratory investigations of the patient's peripheral B-cell sub-populations are expressed as % of CD19+ peripheral B-cells. FASCIA responses are expressed as number of CD4+ T-cell blasts/ µl. IgG antibodies towards Pneumococcus was assessed as a total response against a combination of serotypes (1-5, 6B, F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F). Survival of skin biopsy derived, cultured primary fibroblast exposed to ionizing radiation, numbers are expressed as % surviving cells. Normal values are derived from analysis of fibroblast from a healthy control (HC) analysed at the same time. SEA, Staphylococcal enterotoxin A, PWM, Pokeweed mitogen, ConA, Concanavalin A, PPD Tb, Purified protein derivative Tuberculin, Pnc, Pneumococcus. G, Gray.

Figure 1

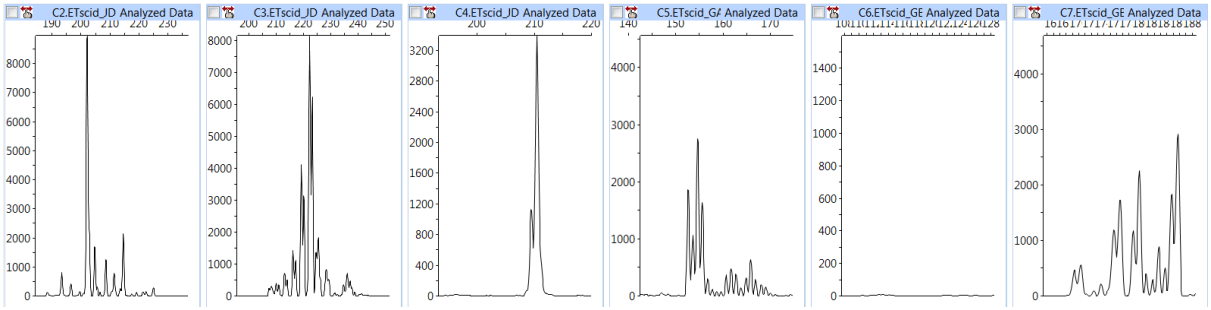
Patient



Control



Patient



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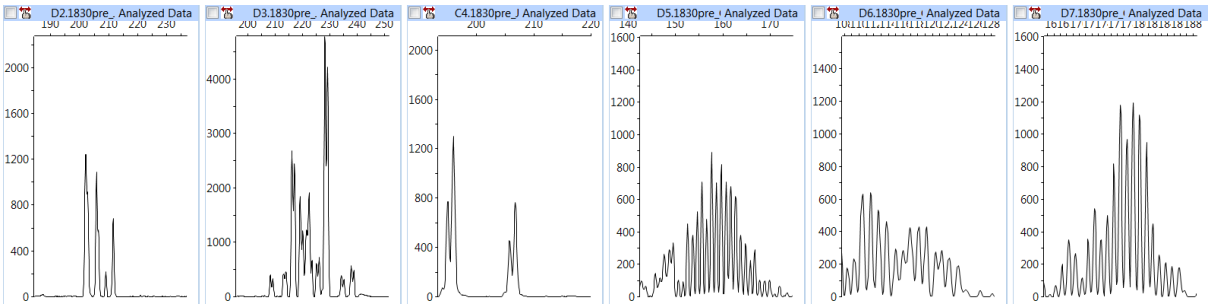

Figure 1. TCR Vβ, Vδ and Vγ CDR3 spectratyping of the patient and a healthy control. The figures present a selection of representative Vβ, Vδ and Vγ families. TCR, T-cell receptor.